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A Complex Chromosome Rearrangement With At Least Five Breakpoints Studied by Fluorescence In Situ Hybridization

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A newborn infant with multiple congenital anomalies was diagnosed with an unbalanced translocation of chromosomes 1 and 5. Studies of parental chromosomes revealed a complex rearrangement in the patient's mother involving the exchange of terminal long arms between chromosomes 1 and 5 and the insertion of an interstitial segment from the same chromosome 5q into chromosome 2q by high-resolution G-banding. Further study of the mother's chromosomes by fluorescent in situ hybridization (FISH) detected an additional insertion between the rearranged chromosomes 2 and 5, which was not revealed by G-banding. This led to the identification of a complex translocation-insertion between 3 chromosomes with at least 5 breaks [t(1;5;2)(1pter→1q42.3::5q23.2→5qter;5pter→5q21.2::2q33→2q35::1q42.3→1qter;2pter→2q33::5q21.2→5q23.2::2q35→2qter)] and illustrates the value of FISH as an adjunct to standard cytogenetics, particularly in cases of complex rearrangements. Am. J. Med. Genet. 68:417–420, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: complex chromosome abnormalities; fluorescence in situ hybridization

INTRODUCTION

Complex chromosome rearrangements (CCR) are rare structural abnormalities often involving more than 2 chromosomes and 3 or more breakpoints [Gorski et al., 1988]. According to the number of chromosome breaks, they are classified as type I (3 or 4 breaks) and type II (5 or more breaks) [Kousseff et al., 1987]. Reported cases have demonstrated as many as 10 breakpoints in such rearrangements [Pai et al., 1980; Fukushima et al., 1986; Kousseff et al., 1987; Till et al., 1991; Tupler et al., 1992]. The characterization of subtle and complicated chromosome abnormalities can be difficult due to the resolution limit of banding analysis. Recently, the application of fluorescence in situ hybridization (FISH) has greatly improved the accuracy of cytogenetic diagnosis and uncovered a number of cryptic aberrations in cases previously studied by conventional cytogenetics [Kuwano et al., 1991; Gandelman et al., 1992]. Here we describe a type II CCR originally identified by high-resolution banding as an unbalanced form in a newborn infant with multiple congenital anomalies. This CCR involving 3 chromosomes was present as a de novo event in the infant's mother and consisted of one translocation and two insertions, of which one was later detected by FISH only.

MATERIALS AND METHODS

A male infant, WM, was born to a normal 28-year-old G2P1011 woman with a history of one prior molar pregnancy. He presented with multiple congenital anomalies, most notably plagiocephalic skull with frontal bossing, hypertelorism, upturned flat nasal bridge, cleft palate, and posteriorly displaced anus. A small atrial septal defect (ASD) was diagnosed and resolved later. Follow-up examinations at 3.5 and 9 months of age showed developmental delay.

Chromosomes for high-resolution G-banding analysis and FISH were prepared from peripheral blood lymphocytes according to standard methods, with 10 μg/ml ethidium bromide added before harvesting. FISH studies using painting libraries specific for chromosomes 1, 2, and 5 (provided by J.W. Gray, University of California, San Francisco) were performed as described previously [Petty et al., 1993]. The painting probes were biotin-labeled and detected by avidin-FITC, and chromosomes were counterstained with propidium iodide. A minimum of 10 metaphase spreads were analyzed for each hybridization using a Zeiss Axiophot epifluorescence microscope.

RESULTS

G-banded chromosomes from the propositus, WM, showed a translocation between chromosomes 1 and 5,

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with a questionable interstitial deletion of 5q material (Fig. 1B, bottom row). High-resolution cytogenetic studies of parental lymphocytes documented a presumably balanced CCR in his mother, DM, with an insertion of 5q material into 2q as well as the same t(1;5) as seen in her son (Fig. 1B, top row). Chromosomes of DM's parents were normal; thus, the presence of this CCR in DM is a de novo event. FISH studies were undertaken to confirm the diagnosis made by G-banding and surprisingly disclosed an additional exchange between the rearranged chromosomes 2 and 5. As shown in Fig. 2B., a hybridization signal on the derivative chromosome 5 was detected by a chromosome 2-specific painting probe, indicating an insertion of a chromosome 2 segment into this chromosome 5. Based on the results of G-banding and FISH studies, the karyotype of DM was interpreted as 46,XX,t(1;5;2)(1pter→1q42.3::5q23.2→5qter;5pter→5q21.2::2q33→2q35::1q42.3→1qter;2pter→2q33::5q21.2→5q23.2::2q35→2qter:). This CCR present in the phenotypically normal mother (DM) contained a reciprocal translocation between the long arms of chromosomes 1 and 5 as well as an exchange of interstitial long arm segments between the chromosome 5 involved in the translocation with chromosome 1 and a chromosome 2. Our patient, WM, having two normal chromosomes 2, inherited only the rearranged chromosomes 1 and 5; thus, he had a duplication of 2q33-q35 and a deletion of 5q21.2-q23.2.

DISCUSSION

The present report illustrates an unbalanced type II CCR inherited from a normal mother with a balanced rearrangement confirmed by FISH. The chromosome complement of the newborn is the least abnormal of any of the possible unbalanced segregants. Some of his phenotypic manifestations are common to many chromosomal disorders and are consistent with other reported cases of similar 5q deletions [Rivera et al., 1990; Lindgren et al., 1992] or 2q duplications [Yu and Chen, 1982].

Individuals carrying balanced CCRs are at increased reproductive risk as most CCR families have been ascertained through children with congenital malformations and/or spontaneous abortuses. Gorski et al. [1988] reviewed 25 CCR families and suggested that general estimates of risk can be applied to all CCR carriers, with the frequency of spontaneous abortion being 46% for female carriers and an 18.4% risk of a liveborn child being abnormal. However, they also stipulated that the size of the rearranged chromosomal segments will alter the risks, as seen in cases of simple reciprocal translocations. Furthermore, Daniel et al. [1988] proposed that when insertional events are part of a CCR, the risk

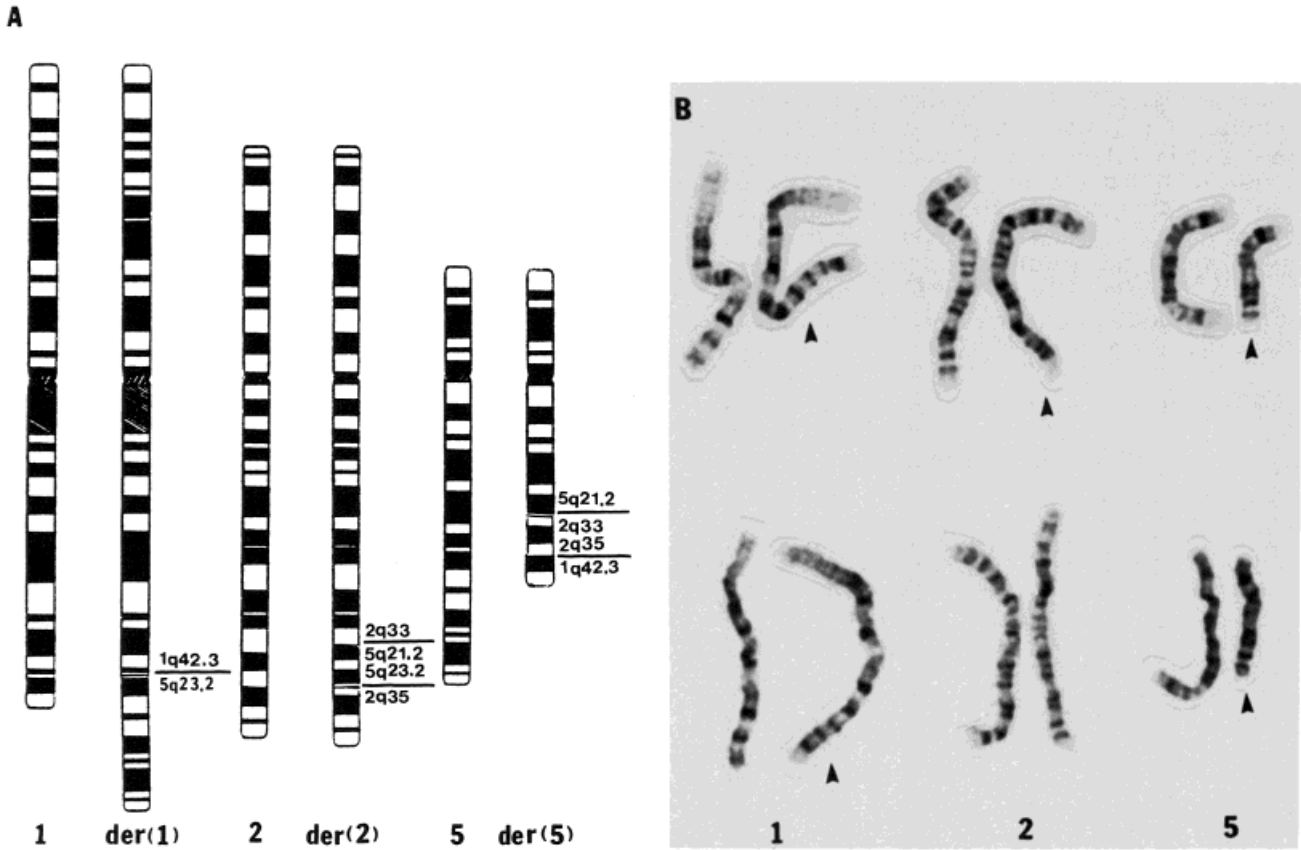

Fig. 1. **A**: Ideogram of normal chromosomes 1, 2, and 5 and their derivatives with lines showing breakpoints. **B**: Partial G-banded karyotype of DM (balanced carrier, top row) and WM (bottom row), with triangles indicating the derivative chromosomes.

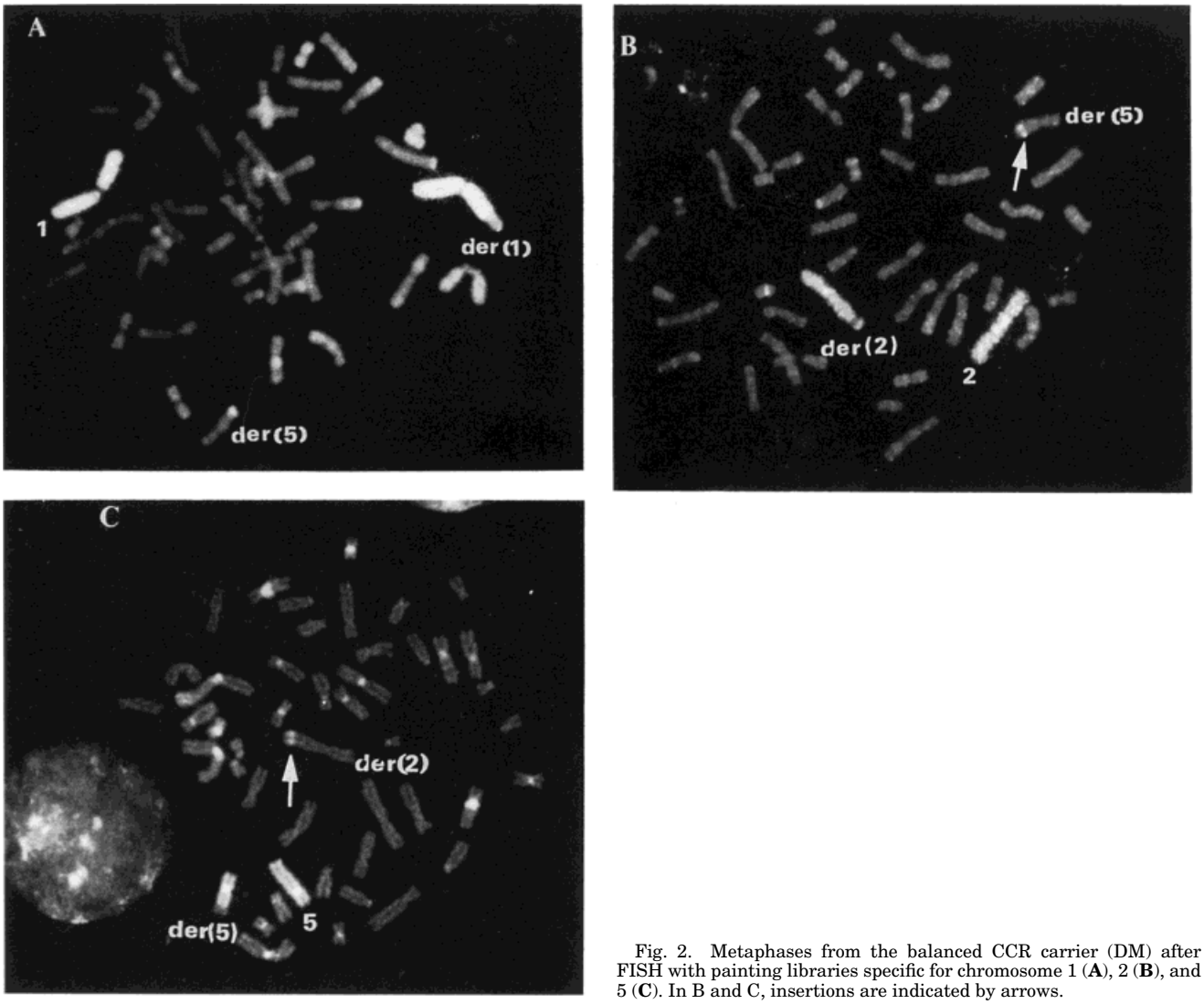

Fig. 2. Metaphases from the balanced CCR carrier (DM) after FISH with painting libraries specific for chromosome 1 (**A**), 2 (**B**), and 5 (**C**). In B and C, insertions are indicated by arrows.

of an unbalanced outcome will be significantly higher. This may well be the case for the CCR carrier described here as there are two insertions involving small chromosomal regions.

CCRs, particularly those of type II, are rare; most are de novo events [Kousseff et al., 1987; Gardner and Sutherland, 1989] and are associated with at least mild malformations, even in balanced form [Lopreiato et al., 1992; Till et al., 1991]. In the familial cases, the transmission of CCRs was found mainly through female carriers and the origin of CCRs was always paternal [Batista et al., 1994]. The unbalanced CCR in our patient (WM) was also inherited from his mother (DM), but the cytogenetic polymorphism, 1qh, of DM's parents was uninformative and did not allow the origin assessment of her CCR. In general, the familial balanced CCR carriers are phenotypically normal. However, in one family described by Handmaker et al. [1975], all the balanced CCR carriers demonstrated similar minor anomalies. Considering the difficulty of precise characterization of CCRs by conventional cytogenetics, the true "balanced" state of certain CCRs could be doubtful. For example, the insertion of 2q material into the rearranged chromosome 5 in our case would not have been identified if not for analysis by FISH.

FISH studies have allowed more precise delineation of this structural abnormality that involves at least 5 chromosome breaks if the breakpoints of the translocation and insertions on chromosomes 2 and 5 indeed coincide molecularly. The size of the rearranged segments and the similarity of G-banding pattern in the regions of 2q34 and 5q23 do not permit accurate identification of the translocation-insertions between them by routine chromosome analysis. FISH identified the duplication of 2q33-q35 in our patient that would have otherwise been misdiagnosed. This case demonstrates further the value of FISH studies in defining complex chromosomal abnormalities and providing the accuracy that is essential for appropriate reproductive risk assessment and genetic counseling.

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